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Abstract

Receptors for purines and pyrimidines are expressed throughout the cardiovascular system. This study investigated their functional expression in porcine-isolated pancreatic arteries. Pancreatic arteries (endothelium intact or denuded) were prepared for isometric tension recording and preconstricted with U46619, a thromboxane A_2 mimetic; adenosine-5′-diphosphate (ADP), uridine-5′-triphosphate (UTP) and MRS2768, a selective $P2Y_2$ agonist, were applied cumulatively, while adenosine-5′-triphosphate (ATP) and αβ-methylene-ATP (αβ-meATP) response curves were generated from single concentrations per tissue segment. Antagonists/enzyme inhibitors were applied prior to U46619 addition. ATP, αβ-meATP, UTP and MRS2768 induced vasoconstriction, with a potency order of αβ-meATP > MRS2768 > ATP ≥ UTP. Contractions to ATP and αβ-meATP were blocked by NF449, a selective P2X1 receptor antagonist. The contraction induced by ATP, but not UTP, was followed by vasorelaxation. Endothelium removal and DUP 697, a cyclooxygenase-2 inhibitor, had no significant effect on contraction to ATP but attenuated that to UTP, indicating actions at distinct receptors. MRS2578, a selective $P2Y_6$ receptor antagonist, had no effect on contractions to UTP. ADP induced endothelium-dependent vasorelaxation which was inhibited by MRS2179, a selective $P2Y_1$ receptor antagonist, or SCH58261, a selective adenosine A_{2A} receptor antagonist. The contractions to ATP and αβ-meATP were attributed to actions at P2X1 receptors on the vascular smooth muscle, whereas it was shown for the first time that UTP induced an endothelium-dependent vasoconstriction which may involve $P2Y_2$ and/or $P2Y_4$ receptors. The relaxation induced by ADP is mediated by $P2Y_1$ and A_{2A} adenosine receptors. Porcine pancreatic arteries appear to lack vasorelaxant $P2Y_2$ and $P2Y_4$ receptors.

Keywords separated by ' - '

αβ-meATP - ATP - UTP - ADP - MRS2578 - $P2Y_1$ - $P2Y_2$ - P2X1 - A_{2A} adenosine receptors - Vasoconstriction - Relaxation - Endothelium

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ORIGINAL ARTICLE

Investigation of the functional expression of purine and pyrimidine receptors in porcine-isolated pancreatic arteries

M. Alsaqati · S. L. F. Chan · V. Ralevic

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Abstract Receptors for purines and pyrimidines are expressed throughout the cardiovascular system. This study investigated their functional expression in porcine-isolated pancreatic arteries. Pancreatic arteries (endothelium intact or denuded) were prepared for isometric tension recording and preconstricted with U46619, a thromboxane A_2 mimetic; adenosine-5′-diphosphate (ADP), uridine-5′-triphosphate (UTP) and MRS2768, a selective $P2Y_2$ agonist, were applied cumulatively, while adenosine-5′-triphosphate (ATP) and αβ-methylene-ATP (αβ-meATP) response curves were generated from single concentrations per tissue segment. Antagonists/enzyme inhibitors were applied prior to U46619 addition. ATP, αβ-meATP, UTP and MRS2768 induced vasoconstriction, with a potency order of αβ-meATP > MRS2768 > ATP ≥ UTP. Contractions to ATP and αβ-meATP were blocked by NF449, a selective P2X1 receptor antagonist. The contraction induced by ATP, but not UTP, was followed by vasorelaxation. Endothelium removal and DUP 697, a cyclooxygenase-2 inhibitor, had no significant effect on contraction to ATP but attenuated that to UTP, indicating actions at distinct receptors. MRS2578, a selective $P2Y_6$ receptor antagonist, had no effect on contractions to UTP. ADP induced endothelium-dependent vasorelaxation which was inhibited by MRS2179, a selective $P2Y_1$ receptor antagonist, or SCH58261, a selective adenosine A_{2A} receptor antagonist. The contractions to ATP and αβ-meATP were attributed to actions at P2X1 receptors on the vascular smooth muscle, whereas it was shown for the first time that UTP induced an endothelium-dependent vasoconstriction which may involve $P2Y_2$ and/or $P2Y_4$ receptors. The relaxation induced by ADP is mediated by $P2Y_1$ and A_{2A} adenosine receptors. Porcine pancreatic arteries appear to lack vasorelaxant $P2Y_2$ and $P2Y_4$ receptors.

Keywords αβ-meATP · ATP · UTP · ADP · MRS2578 · $P2Y_1$ · $P2Y_2$ · P2X1 · A_{2A} adenosine receptors · Vasoconstriction · Relaxation · Endothelium

Abbreviations

αβ-meATP	αβ-Methylene-adenosine-5′-triphosphate
ADP	Adenosine-5′-diphosphate
ATP	Adenosine-5′-triphosphate
EDCFs	Endothelium-derived contractile factors
ENTPDase	Ecto-nucleotidase 5′-triphosphate diphosphohydrolase
PPADS	Pyridoxal phosphate-6-azo(benzene-2,4-disulfonic acid)
UTP	Uridine-5′-triphosphate
VSMCs	Vascular smooth muscle cells
XAC	Xanthine amine congener

Introduction

The activities of both exocrine and endocrine cells of the pancreas are regulated by autonomic nerves (parasympathetic and sympathetic) as well as by hormones and autocrine and paracrine mediators. Although the exact mechanisms remain to be established, it is generally agreed that an increase in endocrine cell activity during hormone secretion corresponds with an increase in blood flow, to meet the metabolic demand. The role of exogenous purine and pyrimidine nucleotides in controlling the functions of endocrine and exocrine components of the pancreas are well described [1, 2], but little is known about their effects on pancreatic arterial vasocontractility.

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There are two main families of P2 purine and pyrimidine receptors, ionotropic P2X and G protein-coupled P2Y receptors. Molecular cloning has identified seven mammalian P2X receptor subunits: P2X1, P2X2, P2X3, P2X4, P2X5, P2X6 and P2X7 [3], while eight mammalian P2Y receptors have been identified: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$ [4]. P2X receptors are activated by adenosine-5′-triphosphate (ATP) and its stable, and consequently more potent, analogue αβ-methylene-ATP (αβ-meATP) [5, 6]. P2Y receptors can be divided on the basis of their endogenous agonists into adenine nucleotide-preferring ($P2Y_1$, $P2Y_{11}$, $P2Y_{12}$ and $P2Y_{13}$) receptors and uracil nucleotide or UDP-sugar-preferring ($P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{14}$) receptors [7]. Among the adenine nucleotide group, the human $P2Y_{11}$ receptor is selectively activated by ATP and fails to respond to adenosine-5′-diphosphate (ADP) [8], although the dog orthologue responds to both ADP and ATP [9]. $P2Y_1$, $P2Y_{12}$ and $P2Y_{13}$ receptors are activated by ADP, with lower potency by ATP [10–13]. Among the uracil nucleotide or UDP-sugar receptors, $P2Y_2$ is equally activated by ATP and uridine-5′-triphosphate (UTP), while $P2Y_4$ receptor is highly selective for UTP over ATP [14]. The $P2Y_6$ receptor is activated by UDP and UTP, while the $P2Y_{14}$ receptor is activated by UDP and UDP-sugars [6, 15].

Within the pancreatic vasculature, P2X1, P2X2, $P2Y_1$ and $P2Y_2$ receptors were detected by immunohistochemistry [16]. More than two decades ago, it was shown that P2X receptors mediate pancreatic vasoconstriction, and P2Y receptors mediate vasodilatation in response to ATP [17], and subsequent studies showed an additional involvement of contractile receptors sensitive to UTP (named P2U receptors) [18]. Purine receptor subclassification has advanced significantly since that time. A re-evaluation of purine receptors in the pancreatic vasculature is clearly warranted. In the current study, we describe the pharmacological characterisation of $P2Y_1$ and A_{2A} receptor-mediated relaxatory responses, in addition to P2X1, $P2Y_2$ and $P2Y_4$ receptor-mediated contractile responses of porcine-isolated pancreatic artery preparations. $P2Y_2$ and/or $P2Y_4$ receptors appear to be expressed mainly in endothelial cells, while P2X1 and A_{2A} receptors appear to be expressed in smooth muscle cells of the pancreatic arteries. A preliminary account of some of these data has previously been presented to the British Pharmacological Society [19].

Materials and methods

Tissue preparation

Pancreases from pigs (either sex, age less than 6 months, wt ~50 kg) were obtained on ice from a local abattoir (G Wood & Sons Ltd., Mansfield). A crude dissection was conducted to isolate the porcine pancreatic arteries (greater pancreatic artery) which were located in the body of the pancreas. The vessels were dissected out and placed in Krebs–Henseleit buffer containing 2 % (*w*/*v*) Ficoll (hydrophilic polysaccharide, type 70) and were refrigerated overnight at 4 °C. The next day, a fine dissection was performed on arteries, and the artery segments were cut into rings of about 0.5 cm in length and suspended in Krebs–Henseleit buffer (gassed, 95 % O_2, 5 % CO_2).

The endothelium of some arteries was removed by gently rubbing the innermost surface of the artery with forceps on a paper tissue before attaching it to the set-up [20]. Successful removal of the endothelium was tested using substance P (10 nM). Endothelium-denuded arteries relaxed in response to substance P to less than 10 % of the U46619-induced contraction, while in endothelium-intact arteries, the relaxation to substance P was 36 %±8 (n =7, data not shown).

Responses in the porcine-isolated pancreatic artery

Arterial rings were mounted onto wires in tissue baths containing warm (37 °C), oxygenated Krebs–Henseleit solution and were connected via isometric force transducers (mechanotransducer MLT 050/D; ADInstruments, Sydney, Australia) to a PC running the computer programme, LabChart (ADInstruments, Sydney, Australia). Rings were put under tension (15 g) and allowed to equilibrate for 60 min before assessing the viability with two challenges of 75 mM potassium chloride (KCl). The tissues were then allowed to equilibrate for 60 min, after which U46619 (10–100 nM), a thromboxane A_2 mimetic, was used to contract the tissues to between 40 and 80 % of the second KCl response. This ensured that if there was a vasodilator component to the response, this could be detected. Once an appropriate level of U46619 response had been achieved, ATP, αβ-meATP, UTP, ADP or MRS2768 were added. Antagonists or enzyme inhibitors were applied 10 min prior to the addition of U46619, allowing them to be incubated with the tissues for a minimal contact time of 30 min prior to the application of agonists. Some arteries were incubated with 0.1 % (*v*/*v*) DMSO (vehicle control).

Reagents and drugs

Krebs–Henseleit buffer was composed of the following (mM): NaCl, 118; KCl, 4.8; $CaCl_2 \cdot H_2O$, 1.3; $NaHCO_3$, 25.0; KH_2PO_4, 1.2; $MgSO_4 \cdot 7H_2O$, 1.2; and glucose, 11.1. Suramin, UTP, ATP, αβ-meATP, ADP, U46619, xanthine amine congener (XAC) and SCH58261 (7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine) were purchased from Sigma (Poole, Dorset, UK), while DUP 697 (5-bromo-2-(4-fluorophenyl)-3-[4-(methylsulfonyl)phenyl]-thiophene), pyridoxal phosphate-6-azo(benzene-2,4-disulfonic acid) (PPADS),

MRS2578 (*N*,*N*″-1,4-butanediyl bis(*N*′-[3-isothiocynatophenyl] thiourea)), MRS2179 (2′-deoxy-N^6-methyladenosine 3′,5′-bisphosphate tetrasodium salt), MRS2768 (uridine-5′-tetraphosphate δ-phenyl ester tetrasodium salt) and substance P were purchased from Tocris Biosciences Ltd. (Bristol, UK). NF449 (4,4′,4″,4′″-[carbonylbis(imino-5,1,3-benzenetriyl-bis(carbonylimino))] tetrakis-1,3-benzenedisulfonic acid) was purchased from Calbiochem-Merck4Biosciences. U46619 was dissolved in ethanol at 10 mM stock concentration. PPADS, suramin, αβ-meATP, ATP, ADP, UTP, NF449, MRS2179, MRS2768 and substance P were dissolved in distilled water. DUP 697, XAC, MRS2578 and SCH58261 were dissolved in DMSO at 10 mM stock concentration.

Data analysis

The contractions to ATP, αβ-meATP and UTP were measured from the stabilised U46619-induced response and were expressed in grams, while the relaxations to ATP and ADP were expressed as a percentage of the U46619-induced contraction. Data were expressed as log concentration–response plots. Values for all figures refer to mean ± standard error of the mean (SEM) with 95 % confidence. Results were compared by one- or two-way ANOVA with Bonferroni's post hoc test or unpaired Student's *t* test (Prism, GraphPad, San Diego, CA, USA). Differences were considered to be significant when the *P* value was <0.05. *N* expresses the number of animals.

Results

Effect of purine and pyrimidine nucleotides on vascular tone in porcine-isolated pancreatic arteries

To investigate the effect of purine and pyrimidine nucleotide agonists on porcine pancreatic arteries, αβ-meATP (10 nM to 100 μM), ATP (1 μM to 10 mM), UTP (10 μM to 1 mM), ADP (1 μM to 1 mM) and MRS2768 (100 nM to 30 μM) were applied after preconstriction with U46619. The responses to ATP and αβ-meATP were found to be desensitised rapidly. Therefore, they were applied at single concentrations (one concentration per tissue segment). The responses to UTP, ADP and MRS2768 did not desensitise rapidly; thus, cumulative concentration–response curves were generated. ATP, αβ-meATP, UTP and MRS2768 induced concentration-dependent contraction with a potency order of αβ-meATP > MRS2768 > ATP ≥ UTP ($P<0.001$, two-way ANOVA; Fig. 1a). The response to ATP was biphasic, since its contraction was followed by a relaxation (Fig. 1b) which was equipotent to the concentration-dependent relaxation produced by ADP (Fig. 1a). The efficacies of ATP and αβ-meATP in inducing contraction were similar and greater than those of UTP or MRS2768. The relaxation to ADP and ATP at the highest concentration of the agonists used (1 mM) was similar at 4.5±0.5 g ($n=10$) and 5.5±0.2 g ($n=7$), respectively; there was no significant difference between these responses (Fig. 1a). UTP, MRS2768 and αβ-meATP did not elicit vasorelaxation.

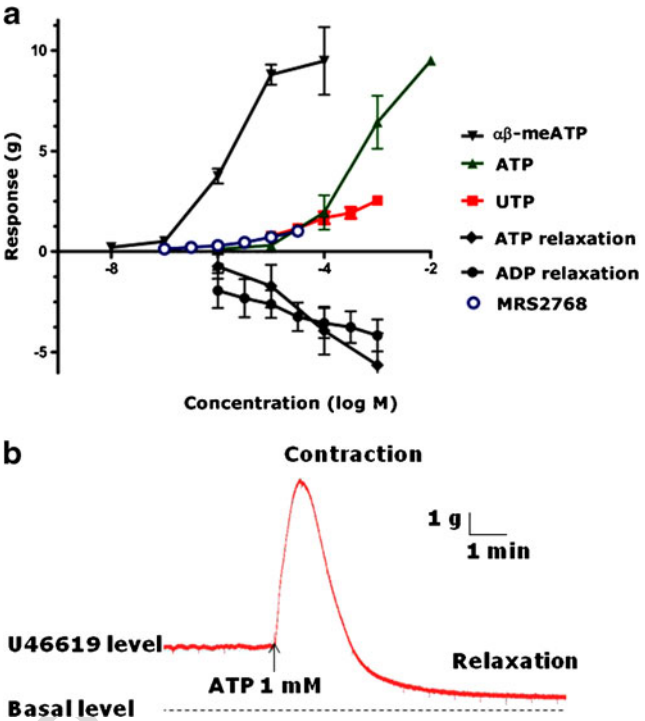

Fig. 1 **a** Concentration-dependent contraction of ATP, αβ-meATP, UTP and MRS2768, a selective $P2Y_2$ agonist, and relaxation of ADP and ATP in U46619-preconstricted porcine pancreatic arteries ($n=7$–12). **b** Typical trace showing the biphasic response to ATP (contraction followed by relaxation). Data are presented as mean ± SEM

Characterisation of responses to ATP and αβ-meATP in U46619-preconstricted porcine-isolated pancreatic arteries

- Effect of suramin, PPADS and αβ-meATP

  Responses to ATP and αβ-meATP were characterised using the non-selective P2 receptor antagonists, suramin (100 μM) and PPADS (10 μM). Both suramin and PPADS significantly attenuated the contractions evoked by ATP (1 mM) and αβ-meATP (1 μM) (Fig. 2a, b). These concentrations of ATP and αβ-meATP were chosen since they produced robust and submaximal responses, and for αβ-meATP, the concentration was close to the half maximal effective concentration (EC_{50}) value [mean EC_{50} value was 1.6 μM (95 % confidence interval (CI), 1.05 to 2.53 μM; $n=8$; Fig. 1a)]. The relaxation to ATP was not affected by suramin or PPADS (Fig. 2c). Since αβ-meATP induces desensitisation of P2X receptors more

readily than ATP because it is broken down more slowly than ATP [5], the responses to ATP and αβ-meATP were studied in the presence of αβ-meATP, in which αβ-meATP (1 μM) was added 10 min prior the addition of U46619. As seen in Fig. 2a, b, the contractions to ATP and αβ-meATP were reduced in the presence of the desensitising agent, while the relaxation to ATP was not affected (Fig. 2c).

- Effect of NF449, a selective P2X1 receptor antagonist

  Contractile responses to αβ-meATP suggest an expression of P2X1 receptors in porcine pancreatic arteries (Fig. 2b). In turn, the involvement of P2X1 receptors in contraction to ATP seems likely because contraction was significantly blocked by αβ-meATP (Fig. 2a). The responses to ATP and αβ-meATP were studied further in the presence of NF449 (10 μM), a P2X1 receptor-selective antagonist. The contractions to ATP and αβ-meATP were inhibited in the presence of NF449 (Fig. 3).
- Effect of endothelium removal

  The response to ATP was tested after the endothelium had been removed. The contraction and the relaxation induced by ATP (Fig. 4) were statistically not significantly different in the absence or presence of the endothelium. Similarly, removal of the endothelium had no effects on the contractions to KCl, U46619 or αβ-meATP; for example, the contraction to 75 mM KCl was 9.5±0.5 g in endothelium-intact arteries, while it was 9±0.5 g in endothelium-denuded arteries (n=7-9). The contraction

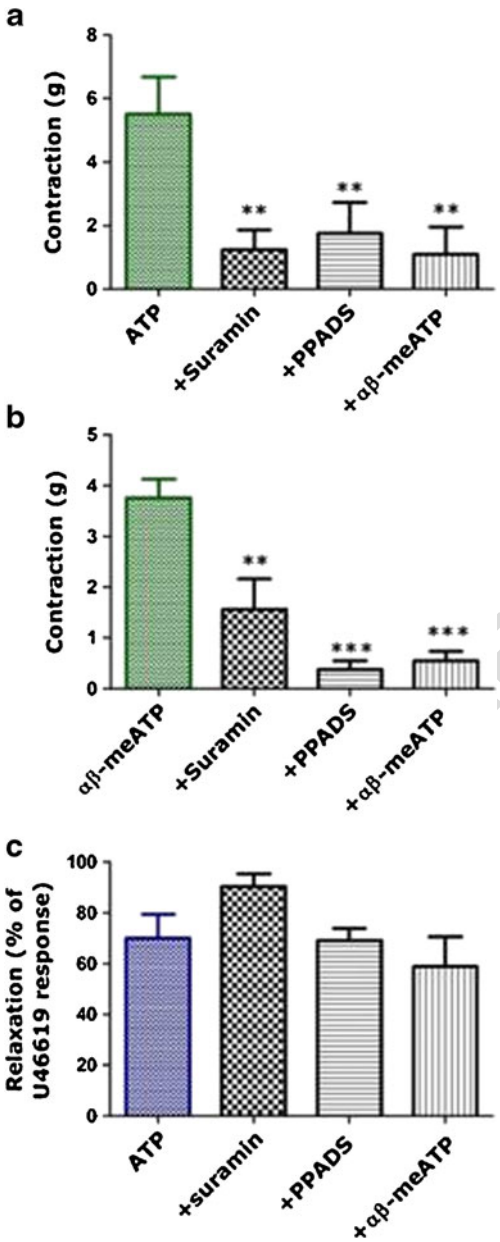

Fig. 2 Effect of suramin (100 μM), PPADS (10 μM) and desensitisation by αβ-meATP (1 μM) on contractions to **a** ATP (1 mM) and **b** αβ-meATP (1 μM) and **c** on the relaxation to ATP in U46619-preconstricted porcine pancreatic arteries. PPADS, suramin and αβ-meATP reduced the contractions of **a** ATP and **b** αβ-meATP (**P<0.01; ***P<0.001, one-way ANOVA with Bonferroni's post hoc test, responses of ATP or αβ-meATP vs their responses in the presence of PPADS, suramin or αβ-meATP, n=6–9). **c** The relaxation to ATP was not significantly different in the absence or presence of PPADS, suramin or αβ-meATP (n=7). Data are presented as mean ± SEM

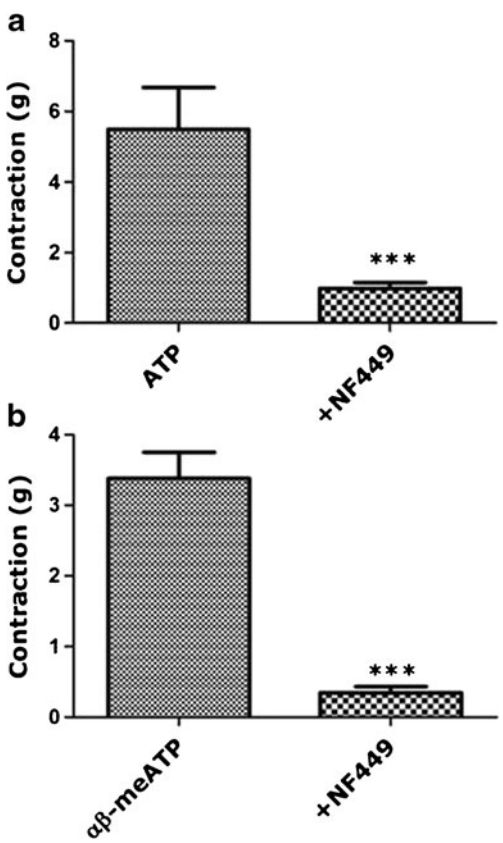

Fig. 3 Effect of NF449 (10 μM), a selective P2X1 receptor antagonist, on contractions to **a** ATP (1 mM) and **b** αβ-meATP (1 μM), in U46619-preconstricted porcine pancreatic arteries. NF449 reduced the effects of **a** ATP and **b** αβ-meATP (***P<0.001, unpaired Student's t test, n=10–13). Data are presented as mean ± SEM

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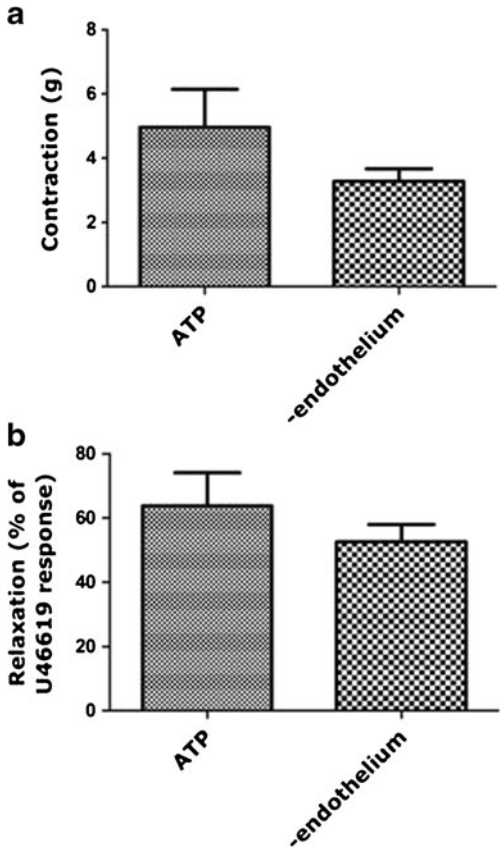

Fig. 4 Effect of removal of the endothelium on **a** contraction and **b** relaxation to ATP (1 mM) in U46619-preconstricted porcine pancreatic arteries. The effect of the removal of endothelium on the contraction or relaxation of ATP was not significantly different (n=9–11). Data are presented as mean ± SEM

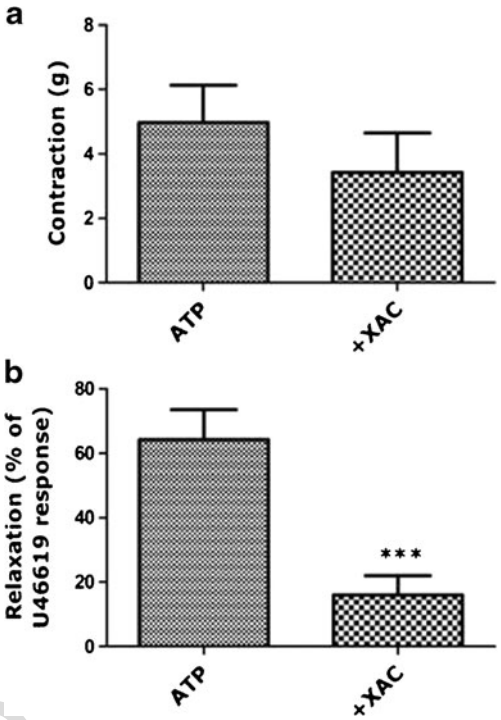

Fig. 5 Effect of XAC (10 μM) on **a** contraction and **b** relaxation to ATP (1 mM) in U46619-preconstricted porcine pancreatic arteries. **a** XAC had no effect on the contraction to ATP (n=8–10), and **b** XAC reduced the relaxation to ATP (***P<0.001, unpaired Student's t test, n=8–10). Data are presented as mean ± SEM

to 10–100 nM U46619 was 5.5±0.5 g in endothelium-intact arteries, while it was 5.8±0.6 g in endothelium-denuded arteries (n=12–14). The contraction to 1 μM αβ-meATP was 3.2±0.6 g in endothelium-intact arteries, while it was 3±0.6 g in endothelium-denuded arteries (n=6); there was no significant difference between these responses.

- Effect of XAC, an adenosine receptor antagonist

  The relaxation to ATP was investigated in the presence of a non-selective adenosine receptor antagonist; XAC (10 μM) had no effect on the contraction evoked by ATP (Fig. 5a), while it reduced significantly the relaxation to ATP (Fig. 5b).

Characterisation of response to UTP in U46619-preconstricted porcine-isolated pancreatic arteries

- Effect of suramin, PPADS, αβ-meATP and MRS2578, a selective $P2Y_6$ receptor antagonist

  The contraction to UTP was examined in the presence of suramin (100 μM), PPADS (10 μM), αβ-meATP (1 μM) and MRS2578 (10 μM). Suramin and PPADS significantly reduced the contraction to UTP (Fig. 6), while the UTP responses were not affected after P2X receptor desensitisation in the presence of αβ-meATP (1 μM) or in the presence of a selective $P2Y_6$ receptor antagonist (MRS2578); for example, the contraction to 1 mM UTP was 1.8±0.2 g in the absence of MRS2578 (n=7), while it was 2.1±0.2 g in the presence of

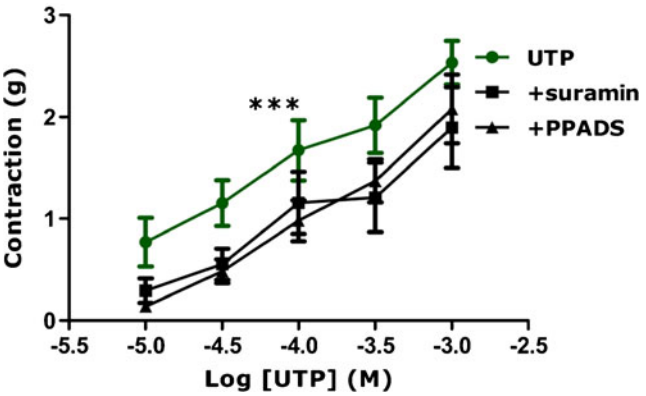

Fig. 6 Effect of suramin (100 μM) and PPADS (10 μM) on contraction to UTP in U46619-preconstricted porcine pancreatic arteries. With suramin and PPADS, effect of UTP concentration (F=16.77 and F=12.38, respectively, ***P<0.001), suramin and PPADS reduced the contraction evoked by UTP (F=14.47 and F=12.48, respectively, ***P<0.001, two-way ANOVA; n=9–12). Data are presented as mean ± SEM

MRS2578 ($n=6$); there was no significant difference between these responses.

- Effect of endothelium removal

  The effects of UTP were studied after the endothelium had been removed. The contraction induced by UTP was significantly attenuated in the endothelium-denuded arteries (Fig. 7).

- Effect of DUP 697, a cyclooxygenase-2 inhibitor

  Because the contraction to UTP was largely endothelium-dependent, the contraction was studied in the presence of DUP 697, a cyclooxygenase-2 (COX-2) inhibitor, since COX-2 facilitates the release of agents which are responsible for endothelium-dependent contraction. DUP 697 (3 μM) diminished the response to UTP (Fig. 8) to a similar extent as removal of the endothelium (Fig. 7), while DUP 697 did not alter the contraction to U46619 (the preconstriction agent) or the contraction to ATP (data not shown).

Characterisation of response to ADP in U46619-preconstricted porcine-isolated pancreatic arteries

- Effect of MRS2179, a $P2Y_1$ receptor selective antagonist, and of endothelium removal

  The relaxation to ADP in pancreatic arteries was studied in the presence of MRS2179 (10 μM) and after the endothelium had been removed. The relaxation to ADP was reduced slightly but significantly in the presence of MRS2179 (Fig. 9a) and in the endothelium-denuded arteries (Fig. 9b), which indicates the involvement of $P2Y_1$ receptors and the endothelium in ADP-mediated relaxation of porcine pancreatic arteries.

- Effect of XAC, an adenosine receptor antagonist, and SCH58261, a selective adenosine A_{2A} receptor antagonist

  The relaxation to ADP was investigated in the presence of XAC (10 μM). The relaxation to ADP was largely reduced in the presence of this inhibitor which indicates the involvement of adenosine receptors (Fig. 10). To find out about the adenosine subtype involved in the relaxation to ADP, the response to ADP was investigated in the presence of SCH58261, a selective adenosine A_{2A} receptor antagonist. This antagonist significantly inhibited the relaxation to ADP, to a similar extent as seen with XAC

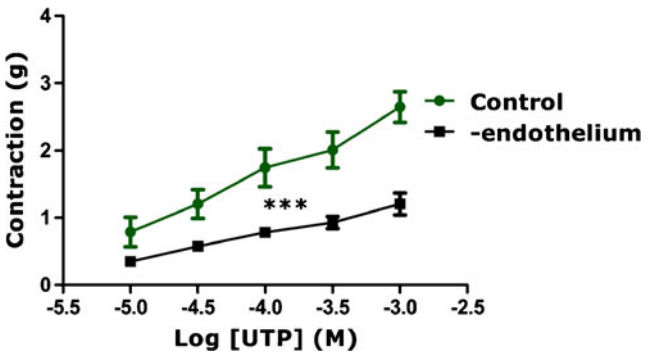

Fig. 7 Effect of removal of the endothelium on contraction to UTP in U46619-preconstricted porcine pancreatic arteries. Effect of UTP concentration ($F=11.91$, $^{***}P<0.001$) and removal of endothelium reduced the contraction evoked by UTP ($F=43$, $^{***}P<0.001$, two-way ANOVA; $n=10$–12). Data are presented as mean ± SEM

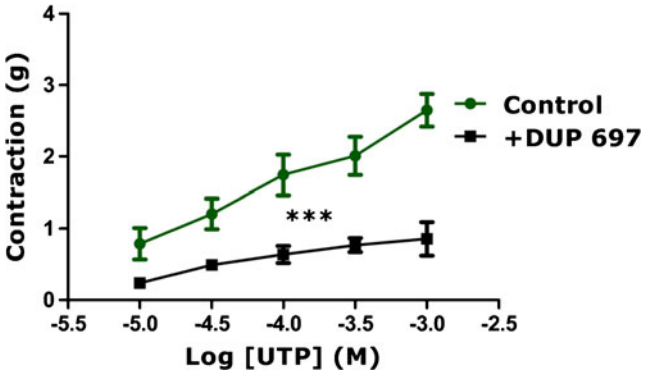

Fig. 8 Effect of DUP 679 (3 μM), a cyclooxygenase-2 inhibitor, on contraction to UTP in U46619-preconstricted porcine pancreatic arteries. Effect of UTP concentration ($F=8.48$, $^{***}P<0.001$) and DUP 679 reduced the contraction evoked by UTP ($F=50.8$, $^{***}P<0.001$, two-way ANOVA; $n=8$–12). Data are presented as mean ± SEM

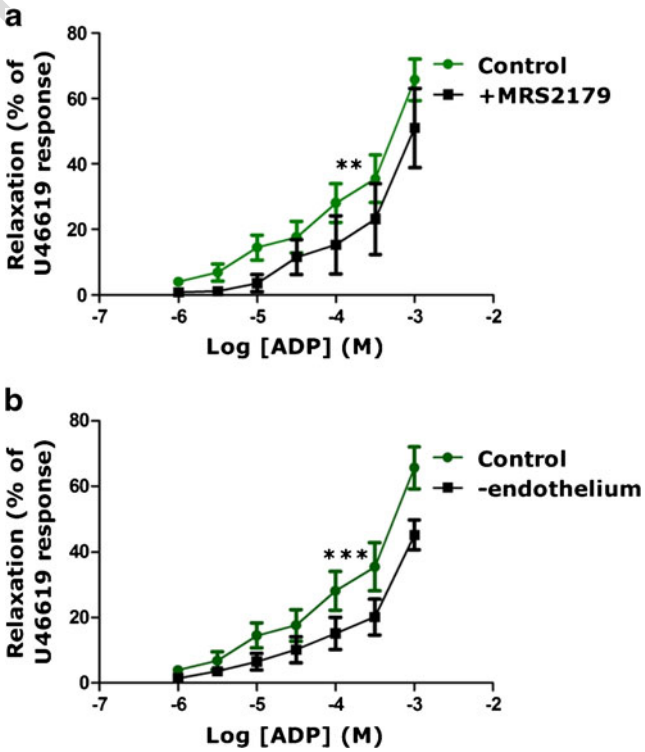

Fig. 9 Effect of **a** MRS2179 (10 μM) and **b** the removal of the endothelium on relaxation to ADP in U46619-preconstricted porcine pancreatic arteries. With MRS2179 and in endothelium-denuded arteries, effect of ADP concentration ($F=21.42$ and 16.77, respectively, $^{***}P<0.001$) and MRS2179 and removal of endothelium reduced the contraction evoked by ADP ($F=21.42$ and $F=32.04$, respectively, $^{***}P<0.001$, two-way ANOVA; $n=10$–12). Data are presented as mean ± SEM

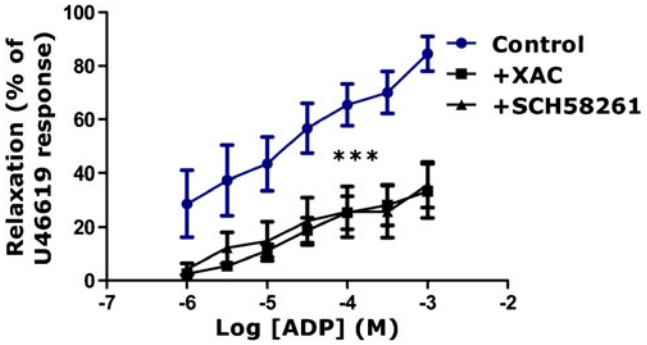

Fig. 10 Effect of XAC (10 μM), a non-selective adenosine receptor antagonist, and SCH58261 (1 μM), a selective adenosine A_{2A} receptor antagonist, on relaxation to ADP in U46619-preconstricted porcine pancreatic arteries. With XAC and SCH58261, effect of ADP concentrations ($F=7.14$ and $F=6.08$, respectively, $^{***}P<0.001$), XAC and SCH58261 reduced the relaxation evoked by ADP ($F=71.19$ and $F=58.16$, respectively, $^{***}P<0.001$, two-way ANOVA; $n=9$–14). Data are presented as mean ± SEM

(Fig. 10). This showed that the relaxation to ADP involved A_{2A} adenosine receptors.

Discussion

The current report has provided evidence for the functional expression of contractile P2X1, $P2Y_2$ and $P2Y_4$ receptors and vasorelaxant $P2Y_1$ and A_{2A} adenosine receptors in porcine pancreatic arteries. These receptors are sensitive to the extracellular nucleotides ATP (P2X1), UTP ($P2Y_2$ and $P2Y_4$) and ADP ($P2Y_1$ and A_{2A}). The contraction to ATP was endothelium independent, while UTP induced an endothelium-dependent contraction which may involve $P2Y_2$ and/or $P2Y_4$ receptors. The relaxation to ADP involved the endothelium and $P2Y_1$ receptors and A_{2A} adenosine receptors.

A vasoconstrictor response elicited by ATP has been reported in a number of different arteries [21–23]. ATP may also induce vasorelaxation depending on the experimental conditions (level of pre-tone) and relative expression of relevant vasocontractile and vasorelaxant receptors [24, 25]. In porcine pancreatic arteries, ATP induced a biphasic response consisting of a contraction followed by a relaxation (Fig. 1b). Since the contraction to ATP was rapidly desensitising, non-cumulative concentration–response curves were investigated. The contractions to ATP and αβ-meATP were reduced in the presence of suramin, PPADS, αβ-meATP (a desensitiser of P2X1 receptors) and NF449 (a P2X1 selective antagonist) (Figs. 2a, b and 3a, b), which indicates that a large part of the contraction to ATP could be attributed to the activation of P2X1 receptors. Moreover, the contractile effect of αβ-meATP is consistent with the expression of P2X1 receptors in porcine pancreatic arteries. αβ-meATP was more potent than ATP in eliciting vasoconstriction most likely due to its greater stability [5]. Since the contraction to ATP was not changed after the endothelium had been removed (Fig. 4a), the expression of P2X1 receptors was shown to be on the vascular smooth muscle cells (VSMCs). This is consistent with the abundant expression of P2X1 receptors on VSMCs of most tissues [7].

ATP-induced vasorelaxation was not affected after the endothelium had been removed or in the presence of suramin or PPADS, which suggests that the relaxation to ATP was not due to its action at P2Y receptors. However, the relaxation to ATP was significantly inhibited in the presence of XAC, which suggested an involvement of adenosine receptors expressed on VSMCs of the pancreatic arteries; it is likely that this is due to the activity of adenosine derived from ATP metabolism by ecto-nucleotidase 5′-triphosphate diphosphohydrolase (ENTPDase) enzymes followed by the activity of CD37 and ecto-5′-nucleotidase enzymes [26]. Similarly, in rat coronary arteries, the relaxation to ATP involved P1 receptors, although there was an additional involvement of P2Y receptors [24]. In the current study, further investigation of the adenosine receptor subtypes involved in the relaxation to ATP is required. We and others have shown previously a slow relaxation in response to αβ-meATP in rat mesenteric arteries, subsequent to contraction [27–29], but we did not observe this in the present study in the porcine pancreatic arteries.

The vasoconstriction to UTP did not desensitise quickly; therefore, cumulative concentration–response curves were used to study the effect of UTP on pancreatic arteries. This contraction was significantly inhibited by suramin and PPADS (Fig. 6), and there was a reduction of the response after the removal of the endothelium (Fig. 7). That would indicate for the first time an endothelium-dependent vasoconstriction evoked by UTP. UTP is known to be active at $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors [30]. The expression of these receptors in the endothelium and smooth muscle of vessels has been reported [31]. Since MRS2578 was not able to alter the contraction to UTP, this indicates that UTP had no action at $P2Y_6$ receptors. There are currently no commercially available selective antagonists for either $P2Y_2$ or $P2Y_4$ receptors. However, we believe that UTP acted at $P2Y_4$ receptors since the contraction to UTP was significantly inhibited by both endothelium removal and in the presence of DUP 697, but responses to ATP were unaffected. UTP-induced contraction may also be mediated by $P2Y_2$ receptors, since MRS2768 which is a selective agonist at $P2Y_2$ receptors and displays no affinity for $P2Y_4$ or $P2Y_6$ receptors was able to evoke a contraction in pancreatic arteries [32] (Fig. 1a).

UTP-induced vasoconstriction has been documented in a number of arteries including rat pulmonary arteries in which the contraction was attributed to $P2Y_2$ receptors, and in rabbit basilar arteries in which the contraction to UTP was due to action of $P2Y_4$ receptors [33, 34]. UTP produced an

endothelium-dependent relaxation in rabbit pulmonary arteries and in rat mesenteric arterial bed, but the receptor subtypes were undefined [22, 35]. In bovine middle cerebral arterial strips, UTP had a dual response, and it induced a contraction in endothelium-denuded arteries, but a relaxation in endothelium-intact arteries [36]. The absence of endothelium-dependent or endothelium-independent relaxation to UTP and some other nucleotides in rat renal arteries was reported [37], which is consistent with the current study since there was no evidence of a UTP-mediated relaxation in porcine pancreatic arteries. Hence, porcine pancreatic arteries appear not to express relaxant $P2Y_2$ and/or $P2Y_4$ receptors.

To investigate the mechanism underlying the contraction mediated by UTP in pancreatic arteries, the response to UTP was examined in the presence of DUP 697. As seen in Fig. 8, the endothelium-dependent contraction was attenuated in the presence of the selective COX-2 inhibitor. Endothelial cells can release endothelium-derived contractile factors (EDCFs), which may include thromboxane A_2, prostaglandin $F_{2\alpha}$, leukotrienes and endothelin-1. Thromboxane A_2 and prostaglandin $F_{2\alpha}$ are released from the endothelium due to the activity of COX-2 [38, 39]. The reduction of the contraction to UTP in the presence of DUP 697 indicated the involvement of thromboxane A_2 and prostaglandins in the contraction to UTP. These agents, after being released from the endothelium, may act on their receptors on VSMCs to cause contraction [39]. The different effects of DUP 697 on responses to UTP and ATP further suggest that they are acting on different receptors.

The relaxation to ADP did not desensitise rapidly; therefore, cumulative concentration–response curves were used to study the effect of ADP on pancreatic arteries. The relaxation was significantly attenuated by MRS2179, a selective $P2Y_1$ receptor antagonist (Fig. 9a). In addition, the relaxation to ADP was reduced after the endothelium had been removed, by a similar extent as observed in the presence of the MRS2179 (Fig. 9b). This may suggest that $P2Y_1$ receptors are expressed on the endothelium. Indeed, a number of reports show that $P2Y_1$ receptors are expressed on the endothelium and are responsible for the relaxation of arteries, including rat thoracic aortic and porcine mesenteric arteries [40, 41]. The relaxation to ADP in our study was largely reduced in the presence of XAC and SCH58261 (adenosine receptor antagonists). Adenosine receptors may be expressed on the endothelium or the vascular smooth muscle [42]. Since XAC and SCH58261 produced a greater reduction in the relaxation to ADP than the inhibition induced by removal of the endothelium (Fig. 10), this suggests that relaxation to ADP involves A_{2A} adenosine receptors expressed, at least in part, on VSMCs. The mechanism by which ADP would produce adenosine to act at the adenosine receptors is still to be elucidated. The simplest explanation is that it is broken down by ENTPDases and by CD37 enzymes to adenosine [26]. Alternatively, as suggested in porcine coronary arteries, ADP mediates a relaxation via a mechanism that involves ADP-evoked adenosine release and the subsequent activation of A_{2A} receptors [20]. In contrast to the porcine pancreatic vessels, ADP in rat pancreatic arteries induced a contraction at a high concentration (1 mM); this contraction was similar to that produced by ATP and was much lower than the contraction induced by αβ-meATP [43]. Further investigation is required to determine the involvement of endothelium-derived relaxing factors or endothelium-derived hyperpolarising factors released from the endothelium in the ADP-induced relaxation.

Reduction in pancreatic blood flow has been observed in acute and chronic pancreatitis and some other pancreatic diseases [44, 45], implicating pancreatic tissue perfusion as an important factor in the pathogenesis of pancreatic diseases and symptoms. There is increasing evidence for the role of purinergic signalling in the pathophysiology of the pancreas [2]. Hence, drugs designed to target specific components of purinergic system may be of relevance to the management of pancreatitis, cystic fibrosis, pancreatic cancer and diabetes.

In summary, the functional expression of P2X1 and A_{2A} adenosine receptors on VSMCs and $P2Y_2$ and/or $P2Y_4$ receptors on the endothelium of porcine pancreatic arteries was indicated in the current study. Activation of P2X1 receptors by ATP or αβ-meATP induced a vasoconstriction, and UTP acts at $P2Y_2$ and/or $P2Y_4$ receptors to induce a contraction. ADP and ATP activate A_{2A} adenosine receptors to induce relaxation, together with an action of ADP on $P2Y_1$ receptors. Pancreatic arteries appear to lack vasorelaxant $P2Y_2$ and/or $P2Y_4$ receptors.

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Q1. Kindly check the inserted spelled out form ‘standard error of the mean’ of the abbreviation ‘SEM’ if correct.
Q2. Kindly check the inserted spelled out form ‘half maximal effective concentration’ of the abbreviation ‘EC_{50}’ if correct.
Q3. Kindly provide access date for references ‘Alsaqati et al. [19]’ and ‘Alefishat et al. [41]’ in the list.